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JAN 16, 2001

GROUP 1600

Date: January 16, 2001

To: Examiner B. Celsa

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From: Carol A. Egnor

Subject: 08/796,164 and 08/874,992

OFFICIALNumber of pages including this cover sheet: 4Please confirm receipt of facsimile: Yes ☐ No ☒**Comments:**

Here is a list of claims allowed in 08/667,003 (Issue Fee received at USPTO 18 October 2000) for you to consider in the related '164 and '992 applications.

Carol

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Allowed Claims in 08/667,003

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Issue Fee received at

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Docket No.: 1818.1010-001
Former Patent No. DUK96-03pA2

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JA:

CLAIMS AS AMENDED IN
AMENDMENT AFTER FINAL REJECTION ON JULY 7, 2000

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35. (Twice Amended) A method for forming a composition comprising polynitrosated methemoglobin, comprising combining methemoglobin with an excess of S-nitrosothiol over methemoglobin in an aqueous solution at a pH of about 7.4 to about 9.2, and maintaining the resulting combination under conditions appropriate for nitrosation to occur at multiple sites on methemoglobin, thereby forming a composition comprising polynitrosated methemoglobin.
36. (Twice Amended) A method for forming a composition comprising polynitrosated or polynitrated oxyhemoglobin in which heme Fe is in the FeII state, comprising combining oxyhemoglobin with an excess of nitrosothiol at a pH of about 7.4 to about 9.2, maintaining the resulting combination under conditions appropriate for nitrosation or nitration to occur, thereby forming a composition comprising polynitrosated or polynitrated methemoglobin, and reacting the polynitrosated or polynitrated methemoglobin with a reagent which selectively reduces FeIII to FeII, thereby forming a composition comprising polynitrosated or polynitrated oxyhemoglobin.
37. (Amended) The method of Claim 36 in which the reagent which selectively reduces FeIII to FeII is a cyanoborohydride.
38. The method of Claim 36 in which the reagent which selectively reduces FeIII to FeII is methemoglobin reductase.

51. A method for producing a composition comprising polynitrosated methemoglobin, comprising incubating oxyhemoglobin with about 100-fold excess S-nitrosoglutathione over protein at about pH 9.2 at about 25°C.
52. A method for producing a composition comprising SNO-methemoglobin having 2 SNO groups per hemoglobin tetramer, comprising incubating oxyhemoglobin with about 100-fold excess S-nitrosocysteine over hemoglobin tetramer, at about pH 7.4 at about 25°C.
54. A method for preparing a composition comprising polynitrosated oxyhemoglobin, said method comprising combining oxyhemoglobin with an excess of S-nitrosocysteine, S-nitrosoglutathione, S-nitrosohomocysteine or S-nitrosocysteinyglycine over hemoglobin in an aqueous solution at a pH of about 7.4 to about 9.2, and maintaining the resulting combination under conditions appropriate for nitrosation to occur at multiple sites on hemoglobin, thereby producing polynitrosated hemoglobin.
55. A method for preparing a composition comprising polynitrosated or polynitrated hemoglobin in which heme Fe is in the FeII state, said method comprising combining hemoglobin with S-nitrosocysteine, S-nitrosoglutathione, S-nitrosohomocysteine or S-nitrosocysteinyglycine at a pH of about 7.4 to about 9.2, maintaining the resulting combination under conditions appropriate for nitrosation or nitration to occur, thereby producing a composition comprising polynitrosated or polynitrated hemoglobin, and polynitrosated or polynitrated methemoglobin, and reacting the polynitrosated or polynitrated methemoglobin with a reagent which selectively reduces FeIII to FeII.

56. A method for preparing a composition comprising polynitrosated methemoglobin, comprising combining oxyhemoglobin with an excess of S-nitrosocysteine, S-nitrosoglutathione, S-nitrosohomocysteine or S-nitrosocysteinylglycine over methemoglobin in an aqueous solution at a pH of about 7.4 to about 9.2, and maintaining the resulting combination under conditions appropriate for nitrosation to occur at multiple sites on methemoglobin, thereby forming a composition comprising polynitrosated methemoglobin.